Crystal Structure of the λ Repressor C-terminal Domain

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Beamline(s): **X12B, X25**

Introduction: The *c*I repressor of bacteriophage λ is a classic example of a protein that binds to its operator DNA sites cooperatively. The C-terminal domain of the repressor mediates dimerization as well as a dimer-dimer interaction that results in the cooperative binding of two repressor dimers to adjacent operator sites. The aim of this study is to determine the structure of the λ repressor C-teriminal domain by x-ray crystallography.

Methods and Materials: The λ repressor C-terminal domain was expressed as an N-terminal 6-His tagged protein in *E. coli* using the pET-14b vector, and purified by Ni-NTA affinity chromatography. High quality crystals were grown in space group $C222_1$ with cell dimensions a=46.0, b=101.3, c=114.3, with two monomers per asymmetric unit, and a solvent content of 55%. A mercury derivative was prepared by soaking for 18 hours in mother liquor plus 2 mM mercuric acetate. A native data set was collected to 1.9Å resolution at beamline X25. Molecular replacement using the model for the UmuD' protein (PDB code 1UMU) was unsuccessful. Using a mercury derivative, multiwavelength anomalous diffraction (MAD) data were collected to 2.5Å at three wavelengths at beamline X12B, and the structure was determined using the program SOLVE.

Results: The structure shows the fold of the monomer is a seven-stranded antiparallel β-sheet followed by a single turn of 3_{10}-helix. The molecular dimer is contained within the asymmetric unit. Combining the structure with a wealth of previous genetic data shows that the dimer-dimer interactions that mediate cooperative binding to adjacent operator sites are captured in the crystal, where two dimers associate about a 2-fold axis of symmetry (Figure 1). The architecture of the tetramer, which has C2 symmetry, is unusual among tetrameric proteins, which typically have D2 or C4 symmetry. As a result, the surface of each dimer buried at the dimer-dimer interface is repeated on the opposite face where it is exposed to solvent. Although only a tetramer is present in the crystal, using the same interactions that bring two dimers together to form a tetramer, two tetramers can be brought together to form an octamer (without steric overlap). However, adding a fifth dimer, to make a decamer, would result in steric clashes. This explains the previous observation that the λ repressor forms octamers (but not higher-ordered oligomers) at high concentrations. Recent data suggest a possible role for the octamer in DNA-looping and gene regulation.

Conclusions: The structure of the λ repressor C-terminal domain provides a model for how two λ repressor dimers are assembled at adjacent operator sites. The structure also explains the unusual oligomerization behavior of the intact repressor in solution.

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References: C. Bell, P. Frescura, A. Hochschild, and M. Lewis, "Crystal Structure of the λ Repressor C-terminal Domain Provides a Model for Cooperative Operator Binding," Cell, 101, 801-811, 2000.

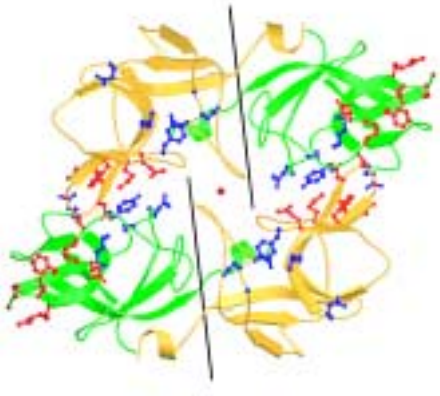

Figure 1. Crystal structure of the λ repressor C-terminal domain. The figure shows how two dimers of the C-terminal domain associate about a 2-fold axis of the crystal (red dot at center). Side chains implicated by genetic studies to be important for cooperativity form two clusters on each monomer, colored red and blue. The association of the two dimers involves the residues identified by the previous genetics, indicating that the interactions responsible for cooperativity have been captured in the crystal.

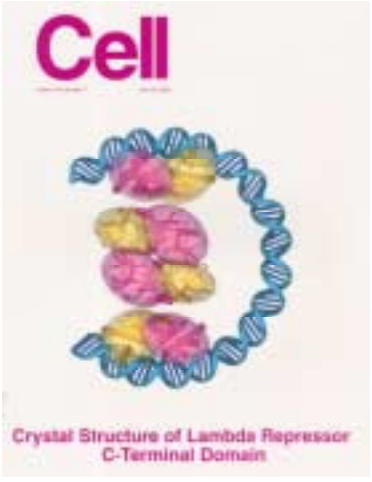

Figure 2. Model for the formation of DNA loops by the λ repressor. In the model, two operator sites are spaced by six turns of duplex DNA. The model combines the structure of the C-terminal domain with the previously determined structure of the N-terminal domain bound to operator DNA.